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AUG 17 2001

TECH CENTER 1600/2900

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Freyssinet, Georges
Perez, Pascal

<120> METHOD FOR OBTAINING PLANT VARIETIES

<130> A33153-PCT-USA 072667.0128

<140> US 09/529,239

<141> 2000-10-27

<150> PCT/EP98/06977

<151> 1998-10-09

<160> 98

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<223> Degenerate oligonucleotides UPMU used to isolate AtMSH3 and AtMSH6.

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<302> Genetics

<303> 132

<306> 963,973

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 <210> 4
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 <212> DNA
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 <210> 5
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Columbia
containing adapter sequences ligated to both its ends

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<210> 7

<211> 29

<212> DNA

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<223> MSH3 specific primer S51 for PCR using cDNA of Arabidopsis
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<210> 8

<211> 24

<212> DNA

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<210> 9

<211> 28

<212> DNA

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ecotype Columbia

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<212> DNA

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<223> MSH3 specific primer 1S5 for PCR using cDNA of Arabidopsis
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<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 52

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360
ccagatgtgg ttttgatggt ggaagttggt tacaggtaca gattcttcgg agaagacgcg
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<210> 14
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 <212> DNA
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 ecotype Columbia

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<210> 15
 <211> 2110
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 <223> Clone 13

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<210> 16
<211> 29
<212> DNA
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thaliana
ecotype Columbia

<400> 16

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<210> 17
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> MSH3 specific primer S525 for PCR using cDNA of Arabidopsis
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ecotype Columbia

<400> 17

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<210> 18
<211> 3522
<212> DNA
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<220>
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<222> (100)...(3342)
<223> AtMSH3 full-length cDNA and deduced sequence of the encoded
polypeptide

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catttctctt aaacggagga gattacgaat aaagcaatt 99

atg ggc aag caa aag cag cag acg att tct cgt ttc ttc gct ccc aaa 147

Met	Gly	Lys	Gln	Lys	Gln	Gln	Thr	Ile	Ser	Arg	Phe	Phe	Ala	Pro	Lys	
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ccc	aaa	tcc	ccg	act	cac	gaa	ccg	aat	ccg	gta	gcc	gaa	tca	tca	aca	195
Pro	Lys	Ser	Pro	Thr	His	Glu	Pro	Asn	Pro	Val	Ala	Glu	Ser	Ser	Thr	
			20					25					30			
ccg	cca	ccg	aag	ata	tcc	gcc	act	gta	tcc	ttc	tct	cct	tcc	aag	cgt	243
Pro	Pro	Pro	Lys	Ile	Ser	Ala	Thr	Val	Ser	Phe	Ser	Pro	Ser	Lys	Arg	
			35				40					45				
aag	ctt	ctc	tcc	gac	cac	ctc	gcc	gcc	gcg	tca	ccc	aaa	aag	cct	aaa	291
Lys	Leu	Leu	Ser	Asp	His	Leu	Ala	Ala	Ala	Ser	Pro	Lys	Lys	Pro	Lys	
	50					55					60					
ctt	tct	cct	cac	act	caa	aac	cca	gta	ccc	gat	ccc	aat	tta	cac	caa	339
Leu	Ser	Pro	His	Thr	Gln	Asn	Pro	Val	Pro	Asp	Pro	Asn	Leu	His	Gln	
	65				70					75					80	
aga	ttt	ctc	cag	aga	ttt	ctg	gaa	ccc	tcg	ccg	gag	gaa	tat	gtt	ccc	387
Arg	Phe	Leu	Gln	Arg	Phe	Leu	Glu	Pro	Ser	Pro	Glu	Glu	Tyr	Val	Pro	
				85					90					95		
gaa	acg	tca	tca	tcg	agg	aaa	tac	aca	cca	ttg	gaa	cag	caa	gtg	gtg	435
Glu	Thr	Ser	Ser	Ser	Arg	Lys	Tyr	Thr	Pro	Leu	Glu	Gln	Gln	Val	Val	
			100					105					110			
gag	cta	aag	agc	aag	tac	cca	gat	gtg	gtt	ttg	atg	gtg	gaa	gtt	ggt	483
Glu	Leu	Lys	Ser	Lys	Tyr	Pro	Asp	Val	Val	Leu	Met	Val	Glu	Val	Gly	
		115					120					125				
tac	agg	tac	aga	ttc	ttc	gga	gaa	gac	gcg	gag	atc	gca	gca	cgc	gtg	531
Tyr	Arg	Tyr	Arg	Phe	Phe	Gly	Glu	Asp	Ala	Glu	Ile	Ala	Ala	Arg	Val	
	130					135					140					
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Leu	Gly	Ile	Tyr	Ala	His	Met	Asp	His	Asn	Phe	Met	Thr	Ala	Ser	Val	
	145				150					155					160	
cca	aca	ttt	cga	ttg	aat	ttc	cat	gtg	aga	aga	ctg	gtg	aat	gca	gga	627
Pro	Thr	Phe	Arg	Leu	Asn	Phe	His	Val	Arg	Arg	Leu	Val	Asn	Ala	Gly	
				165				170					175			
tac	aag	att	ggt	gta	gtg	aag	cag	act	gaa	act	gca	gcc	att	aag	tcc	675
Tyr	Lys	Ile	Gly	Val	Val	Lys	Gln	Thr	Glu	Thr	Ala	Ala	Ile	Lys	Ser	
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His	Gly	Ala	Asn	Arg	Thr	Gly	Pro	Phe	Phe	Arg	Gly	Leu	Ser	Ala	Leu	
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tat	acc	aaa	gcc	acg	ctt	gaa	gcg	gct	gag	gat	ata	agt	ggt	ggt	tgt	771
Tyr	Thr	Lys	Ala	Thr	Leu	Glu	Ala	Ala	Glu	Asp	Ile	Ser	Gly	Gly	Cys	
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ggt	ggt	gaa	gaa	ggt	ttt	ggt	tca	cag	agt	aat	ttc	ttg	gtt	tgt	gtt	819
Gly	Gly	Glu	Glu	Gly	Phe	Gly	Ser	Gln	Ser	Asn	Phe	Leu	Val	Cys	Val	
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gtg	gat	gag	aga	gtt	aag	tcg	gag	aca	tta	ggc	tgt	ggt	att	gaa	atg	867
Val	Asp	Glu	Arg	Val	Lys	Ser	Glu	Thr	Leu	Gly	Cys	Gly	Ile	Glu	Met	

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Ser	Phe	Asp	Val	Arg	Val	Gly	Val	Val	Gly	Val	Glu	Ile	Ser	Thr	Gly															
			260						265						270															
gaa	gtt	gtt	tat	gaa	gag	ttc	aat	gat	aat	ttc	atg	aga	agt	gga	tta	963														
Glu	Val	Val	Tyr	Glu	Glu	Phe	Asn	Asp	Asn	Phe	Met	Arg	Ser	Gly	Leu															
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gag	gct	gtg	att	ttg	agc	ttg	tca	cca	gct	gag	ctg	ttg	ctt	ggc	cag	1011														
Glu	Ala	Val	Ile	Leu	Ser	Leu	Ser	Pro	Ala	Glu	Leu	Leu	Leu	Gly	Gln															
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Gly	Met	Ser	Cys	Leu	Thr	Val	His	Thr	Ile	Met	Asn	Met	Pro	His	Leu															
			370						375						380															
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Thr	Val	Gln	Ala	Leu	Ala	Leu	Thr	Phe	Cys	His	Leu	Lys	Gln	Phe	Gly															
			385						390						395															
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Phe	Glu	Arg	Ile	Leu	Tyr	Gln	Gly	Ala	Ser	Phe	Arg	Ser	Leu	Ser	Ser															
			405						410						415															
aac	aca	gag	atg	act	ctc	tca	gcc	aat	act	ctg	caa	cag	ttg	gag	gtt	1395														
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			435						440						445															
atg	aat	cac	aca	ctt	aca	gta	tat	gct	tcc	agg	ctt	ctt	aga	cac	tgg	1491														
Met	Asn	His	Thr	Leu	Thr	Val	Tyr	Gly	Ser	Arg	Leu	Leu	Arg	His	Trp															
			450						455						460															
gtg	act	cat	cct	cta	tgc	gat	aga	aat	ttg	ata	tct	gct	cgg	ctt	gat	1539														
Val	Thr	His	Pro	Leu	Cys	Asp	Arg	Asn	Leu	Ile	Ser	Ala	Arg	Leu	Asp															
			465						470						475															
gct	gtt	tct	gag	att	tct	gct	tgc	atg	gga	tct	cat	agt	tct	tcc	cag	1587														
Ala	Val	Ser	Glu	Ile	Ser	Ala	Cys	Met	Gly	Ser	His	Ser	Ser	Ser	Gln															
			485						490						495															

ctc	agc	agt	gag	ttg	gtt	gaa	gaa	ggt	tct	gag	aga	gca	att	gta	tca	1635
Leu	Ser	Ser	Glu	Leu	Val	Glu	Glu	Gly	Ser	Glu	Arg	Ala	Ile	Val	Ser	
			500					505					510			
cct	gag	ttt	tat	ctc	gtg	ctc	tcc	tca	gtc	ttg	aca	gct	atg	tct	aga	1683
Pro	Glu	Phe	Tyr	Leu	Val	Leu	Ser	Ser	Val	Leu	Thr	Ala	Met	Ser	Arg	
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tca	tct	gat	att	caa	cgt	gga	ata	aca	aga	atc	ttt	cat	cgg	act	gct	1731
Ser	Ser	Asp	Ile	Gln	Arg	Gly	Ile	Thr	Arg	Ile	Phe	His	Arg	Thr	Ala	
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aaa	gcc	aca	gag	ttc	att	gca	gtt	atg	gaa	gct	att	tta	ctt	gcg	ggg	1779
Lys	Ala	Thr	Glu	Phe	Ile	Ala	Val	Met	Glu	Ala	Ile	Leu	Leu	Ala	Gly	
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Lys	Gln	Ile	Gln	Arg	Leu	Gly	Ile	Lys	Gln	Asp	Ser	Glu	Met	Arg	Ser	
			565					570						575		
atg	caa	tct	gca	act	gtg	cga	tct	act	ctt	ttg	aga	aaa	ttg	att	tct	1875
Met	Gln	Ser	Ala	Thr	Val	Arg	Ser	Thr	Leu	Leu	Arg	Lys	Leu	Ile	Ser	
			580					585					590			
gtt	att	tca	tcc	cct	gtt	gtg	gtt	gac	aat	gcc	gga	aaa	ctt	ctc	tct	1923
Val	Ile	Ser	Ser	Pro	Val	Val	Val	Asp	Asn	Ala	Gly	Lys	Leu	Leu	Ser	
		595					600					605				
gcc	cta	aat	aag	gaa	gcg	gct	gtt	cga	ggt	gac	ttg	ctc	gac	ata	cta	1971
Ala	Leu	Asn	Lys	Glu	Ala	Ala	Val	Arg	Gly	Asp	Leu	Leu	Asp	Ile	Leu	
	610					615					620					
atc	act	tcc	agc	gac	caa	ttt	cct	gag	ctt	gct	gaa	gct	cgc	caa	gca	2019
Ile	Thr	Ser	Ser	Asp	Gln	Phe	Pro	Glu	Leu	Ala	Glu	Ala	Arg	Gln	Ala	
625					630					635					640	
gtt	tta	gtc	atc	agg	gaa	aag	ctg	gat	tcc	tcg	ata	gct	tca	ttt	cgc	2067
Val	Leu	Val	Ile	Arg	Glu	Lys	Leu	Asp	Ser	Ser	Ile	Ala	Ser	Phe	Arg	
			645					650						655		
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Lys	Lys	Leu	Ala	Ile	Arg	Asn	Leu	Glu	Phe	Leu	Gln	Val	Ser	Gly	Ile	
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Thr	His	Leu	Ile	Glu	Leu	Pro	Val	Asp	Ser	Lys	Val	Pro	His	Asn	Trp	
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Val	Lys	Val	Asn	Ser	Thr	Lys	Lys	Thr	Ile	Arg	Tyr	His	Pro	Pro	Glu	
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ata	gta	gct	ggc	ttg	gat	gag	cta	gct	cta	gca	act	gaa	cat	ctt	gcc	2259
Ile	Val	Ala	Gly	Leu	Asp	Glu	Leu	Ala	Leu	Ala	Thr	Glu	His	Leu	Ala	
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att	gtg	aac	cga	gct	tcg	tggt	gat	agt	ttc	ctc	aag	agt	ttc	agt	aga	2307
Ile	Val	Asn	Arg	Ala	Ser	Trp	Asp	Ser	Phe	Leu	Lys	Ser	Phe	Ser	Arg	
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Tyr Tyr Thr Asp Phe Lys Ala Ala Val Gln Ala Leu Ala Ala Leu Asp	
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Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg	
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Pro Glu Phe Val Asp Asp Cys Glu Pro Val Glu Ile Asn Ile Gln Ser	
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Gly Arg His Pro Val Leu Glu Thr Ile Leu Gln Asp Asn Phe Val Pro	
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att tcc ata atg gct cag gtt ggt tcc ttt gta cca gcg tca ttc gcc	2643
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Lys Leu His Val Leu Asp Gly Val Phe Thr Arg Met Gly Ala Ser Asp	
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Gly Ser Tyr Asp His Asp Asp Val Thr Tyr Leu Tyr Lys Leu Val Arg	
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Gly Leu Cys Ser Arg Ser Phe Gly Phe Lys Val Ala Gln Leu Ala Gln
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 Lys Leu Leu Ser Asp His Leu Ala Ala Ala Ser Pro Lys Lys Pro Lys
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 Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln
 65 70 75 80
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 85 90 95
 Glu Thr Ser Ser Ser Arg Lys Tyr Thr Pro Leu Glu Gln Gln Val Val

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Tyr	Arg	Tyr	Arg	Phe	Phe	Gly	Glu	Asp	Ala	Glu	Ile	Ala	Ala	Arg	Val	
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Val	Asp	Glu	Arg	Val	Lys	Ser	Glu	Thr	Leu	Gly	Cys	Gly	Ile	Glu	Met	
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Gly	Met	Ser	Cys	Leu	Thr	Val	His	Thr	Ile	Met	Asn	Met	Pro	His	Leu	
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Phe	Glu	Arg	Ile	Leu	Tyr	Gln	Gly	Ala	Ser	Phe	Arg	Ser	Leu	Ser	Ser	
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Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn
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Met Asn His Thr Leu Thr Val Tyr Gly Ser Arg Leu Leu Arg His Trp
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Val Thr His Pro Leu Cys Asp Arg Asn Leu Ile Ser Ala Arg Leu Asp
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Ala Val Ser Glu Ile Ser Ala Cys Met Gly Ser His Ser Ser Ser Gln
485 490 495
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500 505 510
Pro Glu Phe Tyr Leu Val Leu Ser Ser Val Leu Thr Ala Met Ser Arg
515 520 525
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Lys Ala Thr Glu Phe Ile Ala Val Met Glu Ala Ile Leu Leu Ala Gly
545 550 555 560
Lys Gln Ile Gln Arg Leu Gly Ile Lys Gln Asp Ser Glu Met Arg Ser
565 570 575
Met Gln Ser Ala Thr Val Arg Ser Thr Leu Leu Arg Lys Leu Ile Ser
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Lys Lys Leu Ala Ile Arg Asn Leu Glu Phe Leu Gln Val Ser Gly Ile
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675 680 685
Val Lys Val Asn Ser Thr Lys Lys Thr Ile Arg Tyr His Pro Pro Glu
690 695 700
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740 745 750

Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg
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ecotype Columbia

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ecotype Columbia

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thaliana
ecotype Columbia

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 Phe Gln Lys Pro Thr Ala Ala Thr Thr Lys Gly Leu Val Ser Gly Asp
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gct gct agc ggc ggc ggc ggc agc gga gga cca cga ttt aat gtg aag 267
 Ala Ala Ser Gly Gly Gly Gly Ser Gly Gly Pro Arg Phe Asn Val Arg
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gaa ggc gat gct aaa ggc gac gct tct gta cgt ttt gct gtt tcg aaa 315
 Glu Gly Asp Ala Lys Gly Asp Ala Ser Val Arg Phe Ala Val Ser Lys
 45 50 55

tct gtc gat gag gtt aga gga acg gat act cca ccg gag aag gtt ccg 363
 Ser Val Asp Glu Val Arg Gly Thr Asp Thr Pro Pro Glu Lys Val Pro
 60 65 70

cgt cgt gtc ctg ccg tct gga ttt aag ccg gct gaa tcc gcc gst gat 411
 Arg Arg Val Leu Pro Ser Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp
 75 80 85 90

gct tcg tcc ctg ttc tcc aat att atg cat aag ttt gta aaa gtc gat 459
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Ser	Gly	Arg	Ala	Glu	Leu	Arg	Ser	Val	Glu	Asp	Ile	Gly	Val	Asp	Gly	
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Asp	Val	Pro	Gly	Pro	Glu	Thr	Pro	Gly	Met	Arg	Pro	Arg	Ala	Ser	Arg	
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Pro	Val	Leu	Asp	Ser	Asn	Lys	Arg	Leu	Lys	Met	Leu	Gln	Asp	Pro	Val	
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Glu	Ser	Ser	Arg	Ile	Arg	Asp	Ala	Asn	Arg	Arg	Arg	Pro	Asp	Asp	Pro	
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Met	Ser	Ala	Ser	Gln	Lys	Gln	Tyr	Trp	Ser	Val	Lys	Ser	Glu	Tyr	Met	
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Ser	Gly	Val	Gly	Lys	Cys	Arg	Gln	Val	Gly	Ile	Ser	Glu	Ser	Gly	Ile	
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gat	gag	gca	gtg	caa	aag	cta	tta	gct	cgt	gga	tat	aaa	ggt	gga	cga	1179
Asp	Glu	Ala	Val	Gln	Lys	Leu	Leu	Ala	Arg	Gly	Tyr	Lys	Val	Gly	Arg	
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Ile Glu Gln Leu Glu Thr Ser Asp Gln Ala Lys Ala Arg Gly Ala Asn	
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Thr Ile Ile Pro Arg Lys Leu Val Gln Val Leu Thr Pro Ser Thr Ala	
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Ser Glu Gly Asn Ile Gly Pro Asp Ala Val His Leu Leu Ala Ile Lys	
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Glu Ile Lys Met Glu Leu Gln Lys Cys Ser Thr Val Tyr Gly Phe Ala	
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Phe Val Asp Cys Ala Ala Leu Arg Phe Trp Val Gly Ser Ile Ser Asp	
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Asp Ala Ser Cys Ala Ala Leu Gly Ala Leu Leu Met Gln Val Ser Pro	
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Lys Glu Val Leu Tyr Asp Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys	
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Ala Leu Arg Lys Tyr Thr Leu Thr Gly Ser Thr Ala Val Gln Leu Ala	
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Pro Val Pro Gln Val Met Gly Asp Thr Asp Ala Ala Gly Val Arg Asn	
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Ile Ile Glu Ser Asn Gly Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn	
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Cys Ala Val Asp Gly Leu Asn Glu Cys Asp Val Ala Leu Ser Ala Leu	
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Lys His Gly Asp Ile Phe Pro Tyr Gln Val Tyr Arg Gly Cys Leu Arg	
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Gln	Arg	Val	Lys	Ala	Phe	Gly	Gln	Ile	Val	Lys	Gly	Phe	Arg	Ser	Gly		
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gca	agt	ctc	tct	gct	gga	agc	atg	gcc	agg	cct	ggt	att	ttt	ccc	gaa	2523	
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Pro	Val	Pro	Asn	Asp	Ile	Leu	Leu	Gly	Glu	Ala	Arg	Arg	Ser	Ser	Gly		

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<400> 31

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Asp Ala Ser Val Arg Phe Ala Val Ser Lys Ser Val Asp Glu Val Arg
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Gly Thr Asp Thr Pro Pro Glu Lys Val Pro Arg Arg Val Leu Pro Ser
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Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp Ala Ser Ser Leu Phe Ser
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Asn Ile Met His Lys Phe Val Lys Val Asp Asp Arg Asp Cys Ser Gly
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Glu Arg Ser Arg Glu Asp Val Val Pro Leu Asn Asp Ser Ser Leu Cys
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Met Lys Ala Asn Asp Val Ile Pro Gln Phe Arg Ser Asn Asn Gly Lys
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Thr Gln Glu Arg Asn His Ala Phe Ser Phe Ser Gly Arg Ala Glu Leu
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Arg Ser Val Glu Asp Ile Gly Val Asp Gly Asp Val Pro Gly Pro Glu
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Thr Pro Gly Met Arg Pro Arg Ala Ser Arg Leu Lys Arg Val Leu Glu
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Asp Glu Met Thr Phe Lys Glu Asp Lys Val Pro Val Leu Asp Ser Asn

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Tyr	Phe	Lys	Gly	Ser	Ser	Glu	Ser	Trp	Asn	Cys	Ala	Val	Asp	Gly	Leu
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<210> 32
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 microsatellite

<400> 32

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<210> 33

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of ATHGENEA
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<400> 33

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<210> 34

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer DMCIN-A for PCR on genomic DNA of Arabidopsis
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ssp. Landsberg erecta "Ler"

<400> 34

gaagcgatat tgttcgtg 18

<210> 35

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer DMCIN-B for PCR on genomic DNA of Arabidopsis
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ssp. Landsberg erecta "Ler"

<400> 35

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<210> 36

<211> 31

<212> DNA

<213> Artificial sequence

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<223> Forward primer DMCIN-1 for PCR on genomic DNA of Arabidopsis
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ssp. Landsberg erecta "Ler"

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ssp. *Landsberg erecta* "Ler"

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<210> 39
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<220>
<223> Reverse primer DMCIN-4 for PCR on genomic DNA of *Arabidopsis thaliana*
ssp. *Landsberg erecta* "Ler"

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<223> Forward primer DMC1a for PCR on genomic DNA of *Arabidopsis thaliana* ssp.
Landsberg erecta "Ler"

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<210> 41
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<212> DNA
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<212> DNA
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cgaacagcca acattaattc cc 22

<210> 44
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aaccaaggca cagaagcg 18

<210> 45

<211> 18
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Arabidopsis thaliana subspecies

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<400> 62

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<210> 63

<211> 22

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<213> Artificial sequence

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<223> Reverse primer for PCR amplification of AthBIO2 SSLP marker in Arabidopsis thaliana subspecies

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<223> Forward primer for PCR amplification of AthUBIQUE SSLP marker in Arabidopsis thaliana subspecies

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aggcaaattgt ccatttcatt g 21

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<223> Reverse primer for PCR amplification of AthUBIQUE SSLP marker in Arabidopsis thaliana subspecies

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<210> 66

<211> 21

<212> DNA

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<223> Forward primer for PCR amplification of NGA172 SSLP marker in Arabidopsis thaliana subspecies

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gaaatccaaa tcccagagag g 21

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<400> 82

taccgtcaat ttcacgccc 19

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<210> 84

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<223> Forward primer for PCR amplification of CA72 SSLP marker in
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<400> 84

aatcccagta accaaacaca ca 22

<210> 85

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<223> Reverse primer for PCR amplification of CA72 SSLP marker in
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cccagtctaa ccacgaccac 20

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<223> Forward primer for PCR amplification of NGA151 SSLP marker in
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<210> 89
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<210> 97
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